Cell Metabolism
Perspective

A Ceramide-Centric View of Insulin Resistance

Jose A. Chavez[1,*] and Scott A. Summers[1,2,*]
[1]Sarah W. Stedman Nutrition and Metabolism Center, Duke University Medical Center, Durham, NC 27704, USA
[2]Program in Cardiovascular and Metabolic Diseases, Duke-National University of Singapore Graduate Medical School, Singapore 169857, Republic of Singapore
*Correspondence: jose.chavez@dm.duke.edu (J.A.C.), scott.summers@duke-nus.edu.sg (S.A.S.)
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The recent implementation of genomic and lipidomic approaches has produced a large body of evidence implicating the sphingolipid ceramide in a diverse range of physiological processes and as a critical modulator of cellular stress. In this review, we discuss from a historical perspective the most important discoveries produced over the last decade supporting a role for ceramide and its metabolites in the pathogenesis of insulin resistance and other obesity-associated metabolic diseases. Moreover, we describe how a ceramide-centric view of insulin resistance might be reconciled in the context of other prominent models of nutrient-induced insulin resistance.

Introduction

In 2002, Yusuf Hannun and Lina Obeid penned a review article entitled "The Ceramide-Centric Universe of Lipid-Mediated Cell Regulation: Stress Encounters of the Lipid Kind" (Hannun and Obeid, 2002). The authors noted how advances in lipidomics (i.e., the implementation of mass spectroscopy to quantify lipid species) and genomics (i.e., the cloning of the majority of genes involved in sphingolipid synthesis and degradation) had provided new tools to probe into ceramide function, and that the implementation of such approaches was revealing a potent role for sphingolipids in generalized cellular responses to stress. Indeed, virtually all stress stimuli (e.g., chemotherapeutics, inflammatory agonists, saturated fatty acids, etc.) had by that time been shown to increase rates of ceramide synthesis. Moreover, functional studies in cultured cells were revealing that the lipid had profound effects on cell survival and metabolism. In the years that followed that review, an explosion of published reports using both in vitro and in vivo model systems implicated ceramide accumulation in the pathogenesis of multiple diseases associated with obesity,y including diabetes, cardiomyopathy, and atherosclerosis (Holland and Summers, 2008). Of relevance to this review series, ceramide was shown to contribute to insulin resistance, a metabolic state that places individuals at risk for diabetes and cardiovascular disease (Reaven, 1988).

In this article, we aim to provide a historical perspective of the seminal studies implicating ceramides in insulin resistance and metabolic disease. Moreover, we will discuss how a ceramide-centric view of insulin resistance can be reconciled with other avenues of research linking nutrient oversupply and insulin sensitivity.

A Brief Historical Timeline of Studies Relating Ceramides, Glucosylceramides, and Insulin Resistance

In comparison to the research tools that are available to sphingolipidologists today, approaches for studying sphingolipids in prior decades were somewhat lacking. For example, ceramide quantification most frequently involved a tedious "diacylglycerol-kinase assay" that was viewed by some as not quantitatively reliable. Moreover, this approach is not able to distinguish ceramides from dihydroceramides or provide information on distinct ceramide species. Another limiting factor was the over-reliance of scientists upon exogenous, water-soluble ceramide analogs comprised of a very short acyl chain. These compounds are easy to use experimentally, but have detergent-like properties and were often added at nonphysiological concentrations. Both of these approaches generated a fair bit of concern that biological actions being attributed to the lipid were artifactual in nature (Hofmann and Dixit, 1998; van Blitterswijk et al., 2003). As predicted by Drs. Obeid and Hannun, the advent of lipidomics and genomics allowed for a far more sophisticated analysis of ceramides in cell function. The implementation of these approaches, both in vitro and in vivo, has produced a large body of data implicated ceramide and its metabolites in insulin resistance and metabolic disease. Key discoveries in this area during the last decade include the following:

- Studies using cultured cells and isolated muscles revealed that endogenous ceramides and glucosylceramides antagonize insulin-stimulated glucose uptake and anabolism, and thus could mimic the effects of exogenous sphingolipid analogs.
- Studies in rodent models of obesity revealed that genetic or pharmacological inhibition of ceramide or glucosylceramide biosynthesis is insulin sensitizing.
- Studies in rodents revealed that inhibition of ceramide or glucosylceramide biosynthesis also warded off several pathologies associated with insulin resistance including diabetes, atherosclerosis, hepatic steatosis, and/or cardiomyopathy.
- Studies in cultured cells provided a wealth of mechanistic data about how ceramide impairs insulin signaling.
- Studies applying quantitative lipidomics revealed inverse relationships between ceramide and glucosylceramide levels and insulin sensitivity in both rodents and humans.
- Studies identified strong relationships between inflammatory events, ceramide biosynthesis, and insulin resistance.
- Studies revealed roles for adipokines (e.g., leptin, adiponectin, and tumor necrosis factor alpha) in the modulation of cellular ceramide levels.

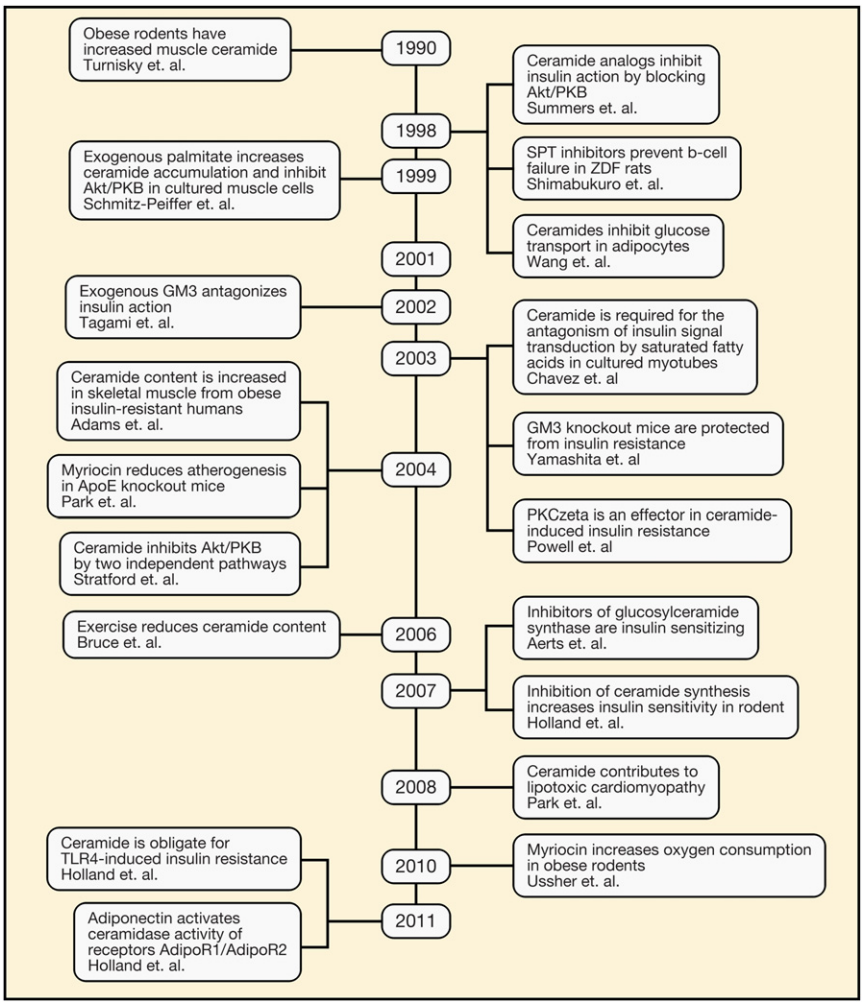

Figure 1. Schematic Timeline of the Seminal Studies Supporting a Role for Ceramides in Insulin Resistance and Metabolic Disease

A large body of literature has accumulated over the past two decades implicating the sphingolipid ceramide and its metabolites in the development of insulin resistance and its associated co-morbidities.

A timeline of the seminal observations is depicted in Figure 1. The net result of these findings is that ceramide is now appreciated as an important nutrient metabolite that accumulates in obesity, altering cellular metabolism and promoting apoptosis, and thus giving rise to many of the hallmark events associated with metabolic disease. These data suggest that inhibitors of ceramide synthesis or activators of ceramide degradation may prove efficacious as therapeutics to combat insulin resistance and metabolic disease.

Cell Culture and Isolated Tissue Studies on Ceramides, Glucosylceramides, and Insulin Resistance

As described above, the first studies evaluating ceramide effects on insulin action involved the use of ceramide analogs that can be added directly to the media bathing cultured cells or isolated tissues without conjugating to carrier proteins. In cultured adipocytes and/or isolated skeletal muscle, these ceramide analogs inhibit insulin-stimulated glucose uptake by blocking translocation of the GLUT4 glucose transporter, as well as glycogen synthesis (Hajduch et al., 2001; Summers et al., 1998; Wang et al., 1998). These effects appear to result from ceramide's ability to block activation of Akt/PKB, a serine/threonine kinase that is obligate for insulin and growth-factor activation of anabolism and cell survival. Overexpression of constitutively active Akt/PKB negates their effects on either apoptosis or glucose uptake (Summers et al., 1998).

Though early reports suggested that ceramide inhibited insulin activation of upstream signaling events (i.e., activation of phosphatidylinositol 3 kinase, which is required for Akt/PKB activation) (Kanety et al., 1996; Peraldi et al., 1996), the majority of studies found no such relationship (Chavez et al., 2003; Hajduch et al., 2001; Kralik et al., 2002; Summers et al., 1998; Teruel et al., 2001; Wang et al., 1998). In all cell types tested, however, ceramide blocks activation of Akt/PKB (Chavez et al., 2003; Powell et al., 2003; Salinas et al., 2000; Stratford et al., 2001; Stratford et al., 2004; Teruel et al., 2001; Zinda et al., 2001). This regulatory event is accomplished by at least two mechanisms (Figure 2):

- First, ceramide blocks the translocation of Akt/PKB to the plasma membrane (Stratford et al., 2001). The Hundal group found that this results from the phosphorylation of Akt/PKB on a regulatory site in the enzyme's PH domain, lowering its affinity for phosphoinositides (Powell et al., 2003). The key intermediate here is the atypical PKC isoform protein kinase Cζ, which is activated by ceramide in vitro (Bourbon et al., 2000). Expression of an Akt/PKB isoform with the S34 site converted to an alanine confers resistance to ceramide in cultured myotubes. Similar findings were obtained in vascular smooth muscle (Bourbon et al., 2002; Fox et al., 2007), where it was further demonstrated that ceramide stabilized interactions between Akt/PKB and PKCζ by recruiting the enzymes to detergent-resistant membrane fractions (e.g., membrane rafts or caveolae) (Fox et al., 2007; Hajduch et al., 2008).
- The second mechanism involves that dephosphorylation of Akt/PKB by protein phosphatase 2A (Chavez et al., 2003; Salinas et al., 2000; Teruel et al., 2001; Zinda et al., 2001). Addition of okadaic acid or overexpression of the SV40 small T antigen, both of which inhibit PP2A, prevents the effects of ceramide on Akt/PKB in a number of different cell types (Chavez et al., 2003; Salinas et al., 2000; Teruel et al., 2001; Zinda et al., 2001).

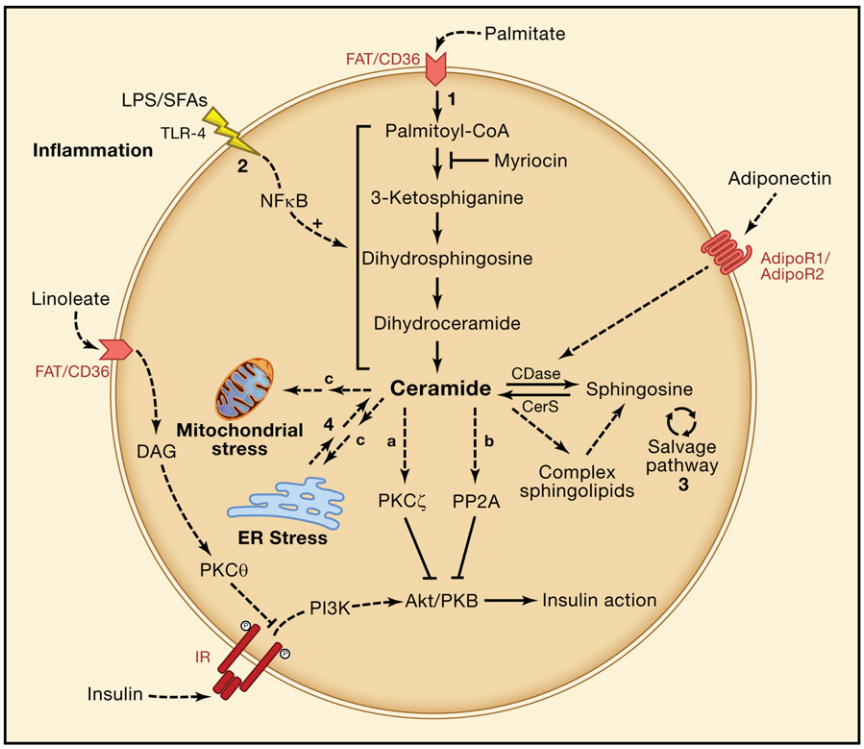

Figure 2. Schematic Diagram Depicting the Multiple mechanisms by which Ceramide Is Generated in Response to Stress Stimuli

Ceramide is generated in response to virtually all stress stimuli including those associated with obesity (e.g., chemotherapeutics, inflammatory agonists, saturated fatty acids, etc.). Cellular ceramide can be formed (1) by a de novo biosynthesis from precursor palmitate; (2) through the activation of inflammatory pathways triggered by TLR4 recognition of saturated fatty acids, which induce the upregulation of genes driving ceramide biosynthesis; (3) through the breakdown of more-complex sphingolipids as part of a "salvage pathway"; and (4) through the disruption of endoplasmic reticulum homeostasis (ER stress). The aberrant accumulation of ceramide may lead to the activation of several signaling and putative targets that may impair normal cellular function, including insulin action. For instance, it has been shown that ceramide (a) directly activates PKCζ isoform which phosphorylates and inhibits the translocation of Akt/PKB; (b) stimulates the activity of a cytosolic protein phosphatase 2A (PP2A), the primary phosphatase responsible for dephosphorylating Akt/PKB; and (c) induce further ER stress and mitochondrial dysfunction. Once generated, ceramide can be metabolized to give rise to a broad array of sphingolipid-derived molecules that may have different effects on cellular function. The insulin sensitizer adiponectin modulates ceramide metabolism via an intrinsic ceramidase activity of its two receptors, AdipoR1 and AdipoR2, thus depleting cells of ceramide and converting it sphingosine, ultimately inducing the formation of the prosurvival factor sphingosine 1-phosphate. DAG produced from polyunsaturated fatty acids (i.e., linoleate) is a potent stimulator PKC isoforms (e.g., PKCθ). This activation was associated with a markedly increment of serine/threonine phosphorylation of IRS-1, a phenomenon that has been shown to reduce its insulin-stimulated tyrosine phosphorylation and downstream propagation of the insulin signaling pathway.

In some cell types, both mechanisms are functional (Dey et al., 2006; Stratford et al., 2004), while in other cultured cell systems either PP2A or PKCζ plays the dominant role (Bourbon et al., 2002; Chavez et al., 2003; Fox et al., 2007). Blouin et al. (2010) proposed that the key determinant of which pathway was dominant in a certain cell type is the relative abundance of caveolae.

As described above, the reliance of the majority of these studies on the water-soluble ceramides cast doubt on the physiological relevance of the ceramide-Akt/PKB relationship. However, subsequent studies revealed that small increases in endogenous ceramide (~50%) were sufficient to inhibit Akt/PKB. The model system most abundantly utilized was developed by Schmitz-Peiffer and Trevor Biden, who demonstrated that exposing cultured myotubes to exogenous palmitate, the most abundant saturated fatty acid in the circulation, increased ceramide accumulation while simultaneously inhibiting Akt/PKB (Schmitz-Peiffer et al., 1999) (Figure 2). The authors concluded that ceramide was the likely intermediate accounting for the antagonism of insulin signaling. Subsequent studies revealed that ceramide was obligate. Using model systems similar to this one, scientists subsequently demonstrated that this increase in endogenous ceramide biosynthesis was requisite for the palmitate effect, as pharmacological inhibition or small interfering RNA (siRNA)-mediated knockdown of enzymes required for ceramide biosynthesis (i.e., serine palmitoyltransferase, ceramide synthases, or dihydroceramide desaturase) completely blocked the palmitate effects on insulin signaling (Chavez et al., 2003; Holland et al., 2011a; Hu et al., 2011; Powell et al., 2003, 2004; Watson et al., 2009). Subsequent studies shown that these approaches also prevented palmitate-antagonism of glucose uptake in isolated soleus muscle preparations from rodents (Holland et al., 2007b) or humans (Thrush et al., 2011).

As an alternative strategy for manipulating endogenous ceramide, we found that blocking rates of ceramide metabolism increased endogenous ceramide levels while blocking activation of Akt/PKB. Specifically, treatment of cultured myotubes with inhibitors of acid ceramidase, which converts ceramide into sphingosine, or glucosylceramide synthase, which converts ceramide into glucosylceramide, exacerbated palmitate-induced insulin resistance (Chavez et al., 2005). Conversely, overexpression of acid ceramidase fully negated the inhibitory effects of palmitate on insulin signaling (Chavez et al., 2003; Powell et al., 2004). These studies confirmed the requirement for ceramide, rather than another sphingolipid metabolite, in the inhibition of Akt/PKB in muscle.

Glucosylceramide is the precursor for a complex family of gangliosides. Unlike cultured myotubes, where glucosylceramides are without effect (J.A.C. and S.A.S., unpublished data), adipocytes are highly sensitive to glucosylated sphingolipids. For example, exogenous GM3 ganglioside inhibits insulin activation of the insulin receptor substrate-1, the first step in signaling cascade linking the hormone to anabolism (Aerts et al., 2007a; Tagami et al., 2002). Moreover, the inflammatory cytokine tumor necrosis factor alpha (TNFα) induces GM3 accumulation in lipid rafts, and the antagonistic effects of the TNFα

can be negated by depleting cells of glucosylated ceramides (Inokuchi, 2006; Kabayama et al., 2005). The mechanism appears to involve the dissociation of insulin receptors from caveolin-1, thus uncoupling them from its downstream signaling effectors (Kabayama et al., 2007). As described below, subsequent in vivo studies confirmed roles for glucosylated ceramides in insulin resistance, adipose inflammation, and hepatic steatosis.

Studies Investigating the Role of Ceramides and Glucosylceramides in Insulin Resistance in Rodents

Manipulation of enzymes controlling sphingolipid synthesis or degradation in mice has a potent effect on insulin resistance and ameliorates lipotoxic responses associated with obesity (Holland and Summers, 2008). The workhorse reagent for these studies is myriocin, a specific inhibitor of serine palmitoyltransferase (SPT), the first and rate-limiting enzyme in the de novo synthesis pathway that converts palmitoyl-CoA and serine into ceramides (Figure 2). Myriocin can be administered chronically to both rats and mice, and it appears to be well tolerated. In rodent models of obesity or other aspects of metabolic disease, myriocin prevents insulin resistance and diabetes (Frangioudakis et al., 2010; Holland et al., 2007a; Ussher et al., 2010; Yang et al., 2009a), atherosclerosis (Glaros et al., 2008; Hojjati et al., 2005; Park et al., 2004, 2008b), and cardiomyopathy (Park et al., 2007, 2008a). Myriocin was originally isolated from an extract of the fruiting bodies of the fungus *Isaria sinclairii* (辛克萊棒束孢) and its parasitic host larva, and has since been identified as a common component in a number of closely related fungal species (e.g., *Cordyceps sinensis*, *Cordyceps cicadea*, and *Cordyceps sinclairii*) (Wang et al., 2009; Yu et al., 2009). Extracts from these species are commonly used in traditional Chinese medicine as a treatment for a plethora of conditions, including diabetes, due to their ability to induce an ''eternal youth'' nostrum. When given to rats, extracts of these agents improve glucose tolerance and insulin sensitivity (Lo et al., 2004, 2006; Paterson, 2008) and ameliorate hypertension (Holloway et al., 2009).

These in vivo studies evaluating the role of ceramide in insulin resistance and diabetes have crossed over a large subset of animal models including the following: high fat-fed mice, *ob/ob* mice, lipid-infused rats, dexamethasone-treated rats and mice, Zucker *fa/fa* rats, and ZDF rats (Frangioudakis et al., 2010; Holland et al., 2007a; Turinsky et al., 1990; Yang et al., 2009b). Hyperinsulinemic-euglycemic clamp studies confirm that myriocin improves insulin sensitivity in both muscle and the liver in these models (Holland et al., 2007a). In the ZDF rats, SPT inhibition additionally preserved pancreatic insulin (William L. Holland and S.A.S., unpublished data) and prevented the onset of frank diabetes (Holland et al., 2007a), confirming studies done by the Unger laboratory using another, albeit less specific, inhibitor SPT inhibitor, cycloserine (Shimabukuro et al., 1998).

Though these findings universally support the enzyme's role in insulin sensitivity in both skeletal muscle and liver, some important differences emerge that warrant elucidation. The relative effects of myriocin on body weight are unclear, and some discrepancies in the literature exist. Two groups, including ours, found that the insulin-sensitizing effects of myriocin were independent of changes in body weight or a reduction in obesity (Holland et al., 2007a; Ussher et al., 2010). Moreover, in apolipoprotein-E-deficient mice ($ApoE^{-/-}$) fed a high-fat diet (discussed below), myriocin also had only a minor impact on body weight (Glaros et al., 2008). However, in a subsequent study using high fat-fed and *ob/ob* mice, myriocin reduced body weight (Yang et al., 2009b). These are important differences that have profound implications on our understanding of the mechanism of sphingolipid action.

Haploinsufficiency for the SPT subunit SPTLC2 and for dihydroceramide desaturase-1 (Des1), both of which are requisite for ceramide biosynthesis, is also insulin sensitizing. Mice heterozygous for SPTLC2 demonstrate reduced peripheral ceramide levels and an improvement in insulin sensitivity after high-fat feeding (Li et al., 2011). Similarly, heterozygous $Des1^{+/-}$ mice are protected from dexamethasone-induced insulin resistance (Holland et al., 2007a).

As mentioned, several laboratories have established a correlation between diets rich in saturated fatty acids with ceramide accumulation and whole-body insulin sensitivity in animals (Blachnio-Zabielska et al., 2010; Frangioudakis et al., 2010; Holland et al., 2007a; Lee et al., 2006). However, to the authors' knowledge, a direct comparison of different saturated fatty acids in the high-fat diet composition with corresponding effects in systemic or tissue ceramide levels and insulin sensitivity is missing.

In mammals, a large family of (dihydro)ceramide synthase (CerS) isoforms exists (these catalyze the acylation of sphinganine to produce dihydroceramide) (Figure 2). At least six genes that encode CerS have been cloned and characterized (Pewzner-Jung et al., 2006). Biochemically, individual CerS isoforms show substrate preference for specific chain length fatty acyl-CoAs, thus generating distinct ceramides with distinct acyl chain lengths. Frangioudakis et al. have recently demonstrated that the expression of CerS1, the most abundant isoform in skeletal muscle involved primarily in the synthesis of C18:0 ceramides, was significantly increased in mice fed a high-fat diet and that this change was associated with alterations in ceramide levels and glucose tolerance (Frangioudakis et al., 2010). However, a detailed study of all ceramide subspecies derived from each CerS isoforms and their implication in the development of insulin resistance has not yet been confirmed through experimentation.

Regarding the glucosylated ceramides discussed above, rodent studies strongly support a role of these metabolites in insulin resistance. First, obesity is associated with enrichment in gangliosides in adipose tissue, as in C57BL/6J, KK, and KKA^{y} mice obesity increases levels of the complex gangliosides GM2, GM1, and GD1a. Second, mice lacking GM3 synthase, which generates the major ganglioside precursor, are protected from insulin resistance and glucose intolerance caused by high-fat feeding (Yamashita et al., 2003). Third, a new generation of highly specific glucosylceramide synthase inhibitors have been shown to improve glucose tolerance and increase insulin sensitivity in muscle and liver of *ob/ob* and DIO mice and ZDF rats, as well as to prevent steatosis and adipose inflammation (Aerts et al., 2007a, 2007b; Bijl et al., 2008, 2009; van Eijk et al., 2009; Zhao et al., 2007, 2009). Collectively, these data strongly suggest that glucosylated ceramides contribute to insulin resistance.

Human Studies Investigating the Role of Ceramides in Muscle Insulin Resistance

Dietary experiments on healthy human subjects to evaluate the effect of high-fat diet enriched with saturated fatty acid (i.e., palmitate) on insulin sensitivity are still lacking. However, since palmitate is the most abundant saturated fatty acid in the diet, we can assume that this experimental approach occurs naturally during obesity. In fact, the associated accruement of ceramides in peripheral tissues of obese insulin resistance subjects has been well documented (Adams et al., 2004). Most studies have been conducted in skeletal muscle (discussed here), though relationships between obesity and ceramide have also been observed in plasma (de Mello et al., 2009; Gill and Sattar, 2009; Haus et al., 2009) and fat (Kolak et al., 2007).

In 2004, Mandarino and colleagues obtained skeletal muscle biopsies from individuals that had undergone a hyperinsulinemic-euglycemic clamp. In this study, total ceramide levels were increased by nearly 2-fold in obese insulin-resistant subjects, and that this was accompanied by a significant reduction in levels of activated Akt/PKB (Adams et al., 2004). In a similar study, Straczkowski et al. performed lipid infusions in human subjects and found a significant negative correlation between ceramide content in skeletal muscle and insulin sensitivity (Straczkowski et al., 2004). A number of subsequent studies demonstrated relationships between muscle ceramide and insulin resistance (Amati et al., 2011; Dubé et al., 2008; Schenk and Horowitz, 2007; Straczkowski et al., 2004, 2007). These observations were further corroborated by interventional studies showing that chronic (Amati et al., 2011; Dubé et al., 2008) or acute (Schenk and Horowitz, 2007) exercise lowered ceramide content in conjunction with an improvement in insulin sensitivity, perhaps by directing lipids into the triglyceride pool. The latter hypothesis is based on recent data obtained by Amati et al. demonstrating that the exercise-induced ceramide reduction was accompanied by an upregulation of several proteins associated with lipid droplet esterification, lipolysis, and oxidation (Amati et al., 2011). In some of these studies, the relationship between ceramide and insulin resistance was in evidence, while a relationship between other lipid species (triglycerides, diacylglycerols) was not supported (Amati et al., 2011; Coen et al., 2010).

To date, no interventional studies have been done on human subjects to evaluate whether pharmacological inhibition of sphingolipid synthesis alters insulin sensitivity.

In contrast to these studies positively associating muscle ceramide with human insulin resistance, some studies have failed to observe such a relationship. For example, Helge and colleagues have consistently observed no relationship between muscle ceramide and insulin sensitivity (Helge et al., 2011a, 2012b; Skovbro et al., 2008). Moreover, Dubé et al. (2011) found that while exercise-induced weight loss improved insulin sensitivity concomitant with a reduction in ceramide, diet-induced weight loss negated insulin resistance without lowering muscle ceramides.

Relationship with Other Models of Nutrient-Induced Insulin Resistance

As will be revealed in this series of review articles, a number of other intracellular intermediates associated with nutrient oversupply have been shown to antagonize insulin action. Importantly, the mechanisms put forth herein and in these other articles are not mutually exclusive, and one would predict that tissues have evolved multiple mechanisms to gauge nutrient status and fuel needs. Indeed, the idea that multiple different nutrient metabolites would alter insulin sensitivity seems not only plausible, but likely. From a personalized medicine perspective, the relative importance of these different nutritional cues would likely have differing levels of importance in distinct subsets of individuals. Nonetheless, careful review of the literature reveals some interesting areas of overlap between the "ceramide hypothesis" and with other nutrient metabolites. Herein we will discuss how a role for ceramide can be reconciled with some of the other pathways discussed in this review series.

Ceramides and Inflammation

Obesity has long been known to associate with a low-grade inflammatory state. One mechanism for this involves the activation of toll-like receptors (TLRs), which induce the transcription of inflammatory cytokines such as TNFα and interleukin-6 (IL6), by saturated fats (Senn, 2006; Shi et al., 2006; Tsukumo et al., 2007). In an elegant series of studies utilizing lipid infusion strategies in TLR4 knockout mice, the Flier group demonstrated the following: (1) saturated fats selectively activate TLRs, (2) TLRs are requisite for lipid induction of TNFα and other cytokines, and (3) knockout mice lacking TLRs are protected from lipid-induced insulin resistance (Shi et al., 2006). TLRs signal through IκKβ and NFκB, and the Shoelson group has identified this pathway as an important contributor to impaired glucose tolerance (Cai et al., 2005; Donath and Shoelson, 2011; Kim et al., 2001; Yuan et al., 2001).

Through the Lipidmaps initiative (http://www.lipidmaps.org), the Merrill group conducted a broad lipidomic profile of cells treated with LPS, a TLR4 agonist. TLR4 activation selectively and strongly increases sphingolipid levels within the cell (Sims et al., 2010). These data suggested that ceramide may be an important effector of TLR4-induced insulin resistance (Figure 2). As demonstrated in Holland et al. (2011a), recent studies revealed that ceramide is indeed an obligate intermediate linking TLR4 to the induction of insulin resistance, both in vitro and in vivo. Moreover, studies in two different patient populations revealed particularly strong correlations between plasma ceramides, circulating cytokines, and insulin resistance (de Mello et al., 2009; Gill and Sattar, 2009; Haus et al., 2009). These data place palmitate, TLR4, and ceramide on a linear pathway that modulates insulin sensitivity (Figure 1).

Ceramides and Mitochondrial Stress

Mitochondrial abnormalities have been seen in some populations of insulin resistance, and the impairment in mitochondrial function has been identified as either a cause or a consequence of the condition (Kraegen et al., 2008; Turner and Heilbronn, 2008). One hypothesis put forth is that the impairment in mitochondrial lipid oxidation leads to the buildup of toxic lipids such as ceramide or diacylglycerol (Bruce et al., 2009). A second idea is that increased flow of nutrients through mitochondrial promotes incomplete fatty acid oxidation and/or oxidative stress, leading to a compensatory impairment in glucose utilization (Koves et al., 2008). Important evidence for this hypothesis involves manipulation of intracellular levels of malonyl-CoA, which inhibits lipid import into mitochondria by blocking carnitine

palmitoyltransferase-1 (CPT1). Depletion of malonyl-CoA decarboxylase (MCD), which leads to the accumulation of malonyl-CoA, increases rates of glucose utilization in vitro (Bouzakri et al., 2008) and in vivo (Koves et al., 2008).

Surprisingly, MCD depletion was associated with a reduction of ceramides (Ussher et al., 2010). One possible explanation for this observation is that the import of acyl-CoAs into the inner mitochondrial membrane may be important for producing particular pools of ceramide, as most of the de novo synthesis have been found on mitochondrial membranes (Bionda et al., 2004; Shimeno et al., 1998). The question then emerges as whether ceramide produced within the organelle could contribute to mitochondrial stress. Depleting ceramides with myriocin in mice fed a high-fat diet improves mitochondrial performance as evidenced by increases in oxygen consumption, increased citrate synthase activity, and preservation of PGC1 expression (Schmitz-Peiffer, 2010; Ussher et al., 2010). The effects of myriocin on lipid oxidation were unclear; long-chain acylcarnitines were increased, suggesting incomplete lipid oxidation, but short-chain acylcarnitines were reduced. Studies in cell culture are also suggestive of roles in mitochondrial stress. As we have reviewed elsewhere, ceramide alters membrane permeability, inhibits electron transport chain intermediates, and promotes oxidative stress (Bikman and Summers, 2011). A full discussion of these myriad actions is beyond the scope of this review, but these findings are generally consistent with idea that the ''mitochondrial stress'' and ''ceramide'' hypotheses are compatible (Figure 2).

Ceramides and ER Stress

Endoplasmic reticulum (ER) stress has also been associated with both β cell apoptosis and peripheral and central insulin resistance that contribute to diabetes (Cnop et al., 2012). Studies in β cell lines implicate ceramide as both a cause (Boslem et al., 2011) and effector (Lei et al., 2010) of ER stress. As saturated fatty acids, but not unsaturated ones, induce ER stress in muscle and liver, ceramide emerged as a likely candidate intermediate linking exogenous lipids disruption of ER function (Figure 2). However, to these authors' knowledge, the only study investigating the role of ceramide in ER stress in insulin sensitive tissues found that inhibitors of ceramide synthesis did not prevent palmitate-induced ER stress in the liver (Wei et al., 2006).

Ceramides and Adipokines

Another idea that has gained some attention is that secretagogues coming from adipose tissue are important modulators of metabolic homeostasis. For example, enlarged adipocytes show increased secretion of the adipokines leptin, resistin, and TNFα, all of which have been implicated in insulin resistance. Moreover, small adipocytes secrete the insulin-sensitizing and cardioprotective hormone adiponectin. Ceramide is implicated in the actions of many of these factors.

- Leptin: Ceramide metabolism appears to play a prominent role in leptin's central control of feeding. Leptin treatment decreases arcuate ceramide levels in mice, and the decrease is important for the hormone's anorectic actions (Gao et al., 2011).
- TNFα: The inflammatory cytokine TNFα) induces GM3 accumulation in lipid rafts, and the antagonistic effects of the TNFα can be negated by depleting cells of glucosylated ceramides (Inokuchi, 2006; Kabayama et al., 2005).
- Adiponectin: Adiponectin receptors are homologous to progestin and adipoQ receptors (PAQR), which were shown in yeast to mediate their actions by activating a ceramidase (Holland and Scherer, 2009; Villa et al., 2009). The homology of these receptors to ceramidase enzymes suggests that this is an intrinsic part of the molecule, and ceramidase inhibitors ablate adiponectin receptor activity (Kupchak et al., 2009). In vivo, Holland et al. recently demonstrated that adiponectin mediates many of its actions by activating a ceramidase activity, thus depleting cells of ceramide and converting it (ultimately) to the pro-survival factor sphingosine 1-phosphate (Holland et al., 2011b) (Figure 2).
- Resistin: A role for ceramide in resistin action has not received much attention. In an isolated muscle system, resistin was shown to blunt insulin action, but only in the presence of palmitate (Junkin et al., 2009). This resistin effect was negated by the inclusion of ceramide synthesis inhibitors. Surprisingly, however, resistin did not increase ceramide levels, and in fact decreased levels of these sphingolipids. Thus, the mechanisms for this interplay are unclear.

Ceramides and the Brain

Recent findings from numerous groups have established an essential role for hypothalamic insulin signaling in the maintenance of glucose tolerance (Gelling et al., 2006; Myers et al., 2009; Obici et al., 2002). Clegg and colleagues demonstrated that the introduction of saturated fatty acids, but not unsaturated ones, directly into the brain disrupted insulin signaling to Akt/PKB in the hypothalamus, altering insulin-regulation of hepatic glucose output (Benoit et al., 2009). The defect was recapitulated in animals fed a high-fat diet (Benoit et al., 2009). We recently found that ceramides accumulate in the hypothalamus of rodents after lipid infusion or high-fat feeding, suggesting that the sphingolipid may have a role in hypothalamic insulin resistance under conditions of nutrient oversupply (Holland et al., 2011a). Thus, while a role for ceramides in the regulation of insulin action in the brain has not been tested directly, it remains a formal possibility.

Ceramides versus DAGs

In addition to ceramides, a number of other lipid intermediates have been suggested to modulate insulin sensitivity. The most prominent of these is diacylglycerol (DAG), which accumulates in the obese and has been suggested to activate protein kinase C (PKC) isoforms that inhibit insulin receptor substrate-1, thus blocking insulin action (Samuel et al., 2010). In cultured myotubes, isolated rodent muscles, or lipid infused rodents, DAG did not appear to contribute to saturated fat (i.e., palmitate)-induced insulin resistance. Indeed, in these model systems, the administration of ceramide synthesis inhibitors restored insulin sensitivity in the face of greatly elevated DAG levels (Chavez et al., 2003; Holland et al., 2007a, 2011a), suggesting that DAG derived from palmitate is a relatively poor inducer of insulin resistance. In these model systems, however, the unsaturated fatty acid linoleate induced insulin resistance through a ceramide-independent mechanism (Holland et al., 2007a),

and DAG is a candidate intermediate for this effect (Figure 2). In fact, infusions of lipid cocktails enriched in linoleate induce insulin resistance and DAG, but not ceramide (Holland et al., 2007a; Itani et al., 2002; Yu et al., 2002). Supportive of these are in vitro experiments showing that unsaturated forms of DAG are more potent activators of PKCs (Hodgkin et al., 1998; Wakelam, 1998). These observations have suggested that DAG may participate in the regulation of insulin signaling by polyunsaturated fatty acids, whereas other derivatives (i.e., ceramides) may be more important for the inhibition of insulin signaling by saturated fatty acids. However, further studies will be needed to address these issues.

Conclusions

Obeid and Hannun were correct in predicting both the ubiquitous role of ceramides in mammalian stress responses and the information explosion that would result from the cloning of genes involved in ceramide metabolism and the increased availability of mass spectroscopy methods to quantify specific ceramide species. Indeed, the "advancing crescendo of studies that have begun to shed significant light on our understanding of ceramide metabolism and function" that they noted in 2002 has shown no signs of diminution. What might not have been so predictable at the time, however, was how new pharmacological approaches and knockout animals would allow for an assessment of the role of ceramide in vivo, and in turn uncover roles in obesity and metabolic disease. The redundancy of approaches utilized to modulate ceramide levels makes it clear that the sphingolipid and/or one of its metabolites plays a quantifiably important role in rodent models of obesity. One can only hope that the rapid pace of progress will ultimately have a translational impact that provides new therapeutic options for those suffering from metabolic disease.

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